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William Ross Hunter[1,2*], Neil Ogle[3]; Nessa O'Connor[2,4].

[1]School of Geography and Environmental Science, Ulster University, Coleraine, BT52 1SA, Northern Ireland (*Present Address*).

[2]Queen's University Marine Laboratory, School of Biological Sciences, Queen's University of Belfast, Portaferry, BT22 1PF, Northern Ireland.

[3]Queen's University Stable Isotope Facility, School of Natural and Built Environment, Queen's University of Belfast, Belfast, BT7 1NN, Northern Ireland.

[4]School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland (*Present Address*).

**Corresponding author: Email: w.hunter@ulster.ac.uk*

Abstract

1. Ocean warming and the loss of larger (often predatory) fauna are major threats to seabed (benthic) ecosystem functioning. Yet we know little about the combined effects of warming and faunal species loss upon the marine carbon cycle.
2. Using stable-isotope pulse-chase experiments, we tested how faunal species loss affects microbial carbon sequestration and retention in intertidal sediments, under both ambient and predicted future warming conditions (ambient + 2°C), using the shore crab *Carcinus maenas* as a model predator. We traced the fixation and retention of a fixed dose of ^{13}C-labelled sodium bicarbonate within sediment organic matter and microbial biomass.
3. *Carcinus* presence was associated with higher total organic carbon concentration within the mesocosm sediments. Temperature had no significant effect upon sediment total organic carbon concentrations. Temperature and *Carcinus* presence had no significant effect on polar lipid fatty acids (PLFAs) concentrations within the sediment, which is a proxy for microbial biomass.
4. *Carcinus* presence increased retention of ^{13}C-labelled carbon within the sediment organic matter pool under future warming conditions. Retention of the ^{13}C-label within the microbial PLFAs decreased significantly under future-warming conditions.
5. Changes in the relative abundance of PLFAs reveals increased contribution of microeukaryotes to the microbial community under ambient conditions, in the absence of *Carcinus*. PLFA profiles revealed significant changes in ^{13}C-label retention within the bacteria and microeukaryotes, driven by interactions between *Carcinus* presence and temperature.
6. Given that temperature is a fundamental control on the metabolic activity of marine organisms (from bacteria to metazoans), we propose that interactions between faunal species loss and ocean warming will have a pronounced effect upon marine carbon budgets.

Key-words Blue Carbon; Carbon Cycle; Carcinus maenas; Faunal loss; Microphytobenthos; PLFA; Predator; Sediment; Stable isotope

1. Introduction

Present estimates indicate that global temperatures will rise by at least 2˚C by the end of the twenty-first century (IPCC 2014). This will have pronounced impacts upon the species distributions, phenology and the metabolic activity of many organisms, in particular those in marine ecosystems (Pauly et al. 1998; Burrows et al. 2011; Bellard et al. 2012; Poloczanska et al. 2013a; Payne et al. 2016). Through its effects upon the metabolic demands of organisms, ocean warming will alter the strength of interactions between autotrophic (primary producer) and heterotrophic (consumer) organisms and affect the rates and pathways for carbon and nutrient cycling (Daufresne, Lengfellner & Sommer 2009; López-Urrutia et al. 2009; O'Connor et al. 2009). Metabolic theory predicts that the influence of temperature upon organismal metabolism is negatively associated with body-size (Ernest et al. 2003). Consequently, microbial processes that dominate carbon and nutrient cycling in marine sediments are predicted to be particularly sensitive to ocean warming (Gillooly et al. 2001; Weston & Joye 2005; Daufresne et al. 2009; Morán et al. 2015).

Current rates of marine species loss are comparable with past mass extinctions, although the present situation is unique in disproportionately affecting larger organisms living at the seabed (Payne et al. 2016). These benthic fauna are functionally important as predators and ecosystem engineers (Jones, C. G., Lawton & Shachak 1994; O'Connor et al. 2009; van Nugteren, Herman et al. 2009; Atwood et al. 2013; O'Connor et al. 2013). Faunal activity has cascading effects through benthic food-webs potentially affecting organic matter preservation (Spivak et al. 2007; Jeffreys, Rachel M., Wolff & Murty 2009; Fanjul et al. 2015), microbial activity (e.g. van Nugteren, Herman et al. 2009; Hunter, Veuger & Witte 2012; Hunter et al. 2013) the trophic transfer of carbon and other nutrients (e.g. O'Connor et al. 2009; O'Connor et al. 2013; O'Connor & Donohue 2013; Atwood et al. 2013) and, the energetic demands of seafloor ecosystems (Piepenburg et al. 1995). Larger predatory fauna, such as crabs, typically exhibit patchy spatial distributions at the seafloor and are highly mobile (Jones, D. O. B. et al. 2014; Atwood et al. 2015; Yool et al. 2017). To counter this, experimental manipulations provide a robust methodology to test how the loss of large fauna affects ecosystem functioning under different environmental contexts (Canuel et al. 2007; Spivak et al. 2007; O'Connor et al. 2013; O'Connor & Donohue 2013).

Non-vegetated coastal sediments are important to the global carbon cycle, accounting for between 13 and 28 % of total marine carbon burial [~ 226 - 418 Tg C yr^{-1}] (Cai 2011; Bauer et al. 2013; Atwood et al. 2015). Over the past 50 years, their areal coverage has increased by between 25 and 50%, driven by changes in land use, climate change and nutrient enrichment (Atwood et al. 2015). In coastal sediments, the microphytobenthos (MPB) are an assemblage of unicellular eukaryotic algae and cyanobacteria that grow in the upper millimetres of illuminated sediments (MacIntyre, Geider & Miller 1996). As such, the MPB are the main primary producers in non-vegetated sediments, contributing up to 50% of the primary productivity in coastal ecosystems (Underwood, G. J. C. & Kronkamp 1999). The MPB play a critical role in carbon fixation and storage in the sediment organic matter pool (Middelburg et al. 2000; Evrard et al. 2010), provide a food source for benthic fauna (Miller, Geider & MacIntyre 1996; Middelburg et al. 2000; Evrard et al. 2010) and, produce extracellular polysaccharides that contribute to sediment stabilisation (Miller et al. 1996; Tolhurst et al. 2006; Spears et al. 2008) and provide a labile organic matter source for heterotrophic microorganisms (Oakes & Eyre 2014). As the area of non-vegetated coastal sediments continues to grow, their responses to the cumulative impacts of faunal species loss and ocean warming will have major implications for carbon cycling in the future ocean.

The impacts of environmental change upon ecosystem processes remains a ecological 'black box' (Burrows et al. 2011; Cardinale et al. 2012; Poloczanska et al. 2013b; Halpern et al. 2015). Stable-isotope labelling experiments provide a powerful tool to open the box, by empirically tracing carbon and energy flow through a biological systems (Middelburg et al. 2000; Boschker, Kromkamp & Middelburg 2005; van Nugteren et al. 2009; Evrard et al. 2010; Hunter et al. 2012; Mayor, Thornton & Zuur 2012; Hunter et al. 2013). By tracing the incorporation and transfer of a carbon source enriched in the rare stable isotope carbon-13 (^{13}C), over a fixed time period, we can test how environmental changes affect various aspects of the carbon cycle, such as carbon fixation and retention (Middelburg et al. 2000; Evrard et al. 2010) or the pathways for organic matter degradation (van Nugteren et al. 2009; van Nugteren, Moodley et al. 2009; Hunter et al. 2012; Mayor et al. 2012; Hunter et al. 2013). In this study, we test how the presence of a benthic predator affected carbon fixation and retention rates in coastal sediments and, whether these putative effects differ between

present climatic conditions and predicted future warming (ambient + 2 ^{o}C) conditions (IPCC 2014). We focus upon the impacts of the shore crab *Carcinas maenas* (Linnaeus, 1758) [hereafter *Carcinus*] a globally distributed predator / scavenger, commonly found on both soft and hard substrata in coastal habitats (Crothers 1968). *Carcinus* is an active bioturbator that excavates soft sediments, transporting buried sediment back to the surface (Queiros et al. 2013). We predict that the effects of *Carcinus* upon carbon fixation and retention within coastal sediments will be mediated by ocean warming. We hypothesise that under ambient conditions, physical disturbance of the sediment surface by *Carcinus* limit the carbon fixation by the microphytobenthos, reducing ^{13}C-retention within the sediment organic matter pool. Based on previous studies (Canuel et al. 2007; Spivak et al. 2007; Fanjul et al. 2015) we hypothesise that *Carcinus* presence will promote the preservation of non-isotopically labelled organic matter within the sediment and limit the accumulation of heterotrophic microbial biomass. As ocean warming is predicted to increase both faunal and microbial metabolic demands, we hypothesise that under future warming conditions (ambient + 2ºC) the direct (and indirect) effects of *Carcinus* presence upon carbon fixation and retention within coastal sediments will be amplified.

2. Materials and Methods

2.1 Experimental Design and Set-up

Stable isotope (^{13}C) pulse-chase experiments were conducted during February and March 2015 using outdoor flow-through mesocosms at Queen's University Marine Laboratory (Portaferry, Northern Ireland (Mrowicki & O'Connor 2015)). We assembled 20 mesocosms containing intertidal sediment manipulating two factors: 1. Presence of *Carcinus* [two levels, present (single crab) and absent] and 2. temperature [two levels, ambient and warming (ambient + 2°C)]. Mesocosms were arranged across four outdoor water baths, supplied with a constant flow of sand-filtered water from the adjacent Strangford Lough [flow rate = 27.84 (± 4.89) l. h^{-1}] and enclosed with plastic mesh lids, with a mesh size of 5 mm, to prevent the crabs from escaping. Elevated temperature treatments were maintained using aquarium heaters (Elite Submersible 300W, Hagen Inc., USA) to warm two of the water baths. Mesocosms received a daily water change, leaving the sediment for 4 hours to simulate low tide. Prior to the daily water change, temperature, salinity, dissolved oxygen and pH were

recorded within each mesocosm using a YSI 6-series sonde and multi-meter [YSI Inc. Ohio, USA] (*Supplementary Table 1*).

Sediment and *Carcinus* were collected from the Dorn mudflats, Strangford Lough (54° 25' 44" N; 5° 32' 34" W) between the 2nd–6th February 2015. Sediments (less than 10 cm depth) were collected, homogenised and packed to 10 cm depth, in twenty 45 L opaque polypropylene boxes "mesocosms" (with internal dimensions 55.5 x 35.5 x 22.0 cm). Collected sediments were a fine muddy-sand composed of 4.40 ± 1.69 % coarse sand (> 1000 um), 69.22 ± 3.20 % fine sand (> 63 um) and 26.37 ± 2.69 % silt (< 63 um), with a sand: silt ratio of 2.85 ± 0.25. Sediment pore water content was 35.29 ± 0.87 % and porosity was calculated as 0.04 ± 0.01.

Experiments were conducted over 4 weeks (28 days). A single male crab [carapace width = 3.47 (± 0.14) cm, wet biomass = 9.66 (± 2.21) g] placed into five ambient and five warmed mesocosms 7 days after the incubations commenced; a 24 mg (0.1 $g.m^{-2}$) dose of ^{13}C-labelled sodium bicarbonate was sprayed directly onto the sediment surface of all mesocosms on day 21. The experiments were then incubated for a further 5 days and then destructively sampled to quantify ^{13}C-incorporation and retention in microbial biomass and the sediment organic matter pool, following Middelburg et al. (Middelburg et al. 2000).

Changes in the biomass and spatial variability of the sediment microphytobenthos (MPB) were measured within each mesocosm using a BBE Moldänke BenthoTorch [BBE Moldänke Gmbh, Schwentinental, Germany] to quantify surficial chlorophyll a (Chl_a) concentrations after 7 (Week 0), 14 (Week 1), 21 (Week 2) and 28 (Week 3) days (Kahlert & McKie 2014). At each interval five replicate measurements of the surficial Chl_a were made, within each mesocosm. Mean Chl_a concentrations within each mesocosm were estimated as a proxy for MPB biomass, with the coefficient of variation of the Chl_a measurements within each mesocosm providing a metric for MPB patchiness within each mesocosm.

After 28 days, the mesocosm sediments were randomly sampled using four 8 cm diameter push cores. These were horizontally sectioned into 0-0.5; 0.5-2; 2-5; 5-10 cm depth fractions. Depth fractions from each of the four cores were pooled, homogenised and lyophilised (-68 °C; 0.0001 mbar), then stored frozen (-20 °C) prior to further analysis (Hunter et al. 2012; Hunter et al. 2013). Total organic carbon

(TOC) and the retention of the ^{13}C-label into the sediment organic carbon pool were determined using a Thermo Flash1112 Elemental Analyser interfaced with a Delta V Isotope Ratio Mass Spectrometer [EA-IRMS]. Analytical precision, based on regular replicated analysis of two international standards (USGS 41 and CH7) was < ±0.1 ‰. Prior to analysis, sediment samples were lyophilised, acid fumed with 6 mol l^{-1} hydrochloric acid to remove inorganic carbon following Hedges and Stern (1984) and dried to constant weight at 60 °C.

Microbial biomass and retention of the ^{13}C-label within the surficial (0-0.5 cm) sediments were quantified, using polar lipid fatty acids (PLFAs) as biomarkers. PLFAs were extracted from 3 g of lyophilized sediment using a modified Bligh-Dyer extraction protocol (White et al. 1979). Briefly, lipids were extracted over 2 hours using a single-phase extraction mixture of chloroform, methanol and citrate buffer (1:2:0.8 *v/v/v*). Lipid extracts were fractionated on silicic acid columns (6 ml ISOLUTE SIS PE columns, International Sorbent Technologies Ltd, Ystrad Mynach, UK) via sequential elution with chloroform (neutral lipid fatty acids), acetone (glycolipid fatty acids) and methanol (PLFAs). PLFAs were transmethylated to fatty acid methyl esters for analysis. PLFA concentrations and $^{13}C/^{12}C$-ratios were determined on a Thermo GC Trace Ultra gas chromatograph combustion interfaced to a Thermo Delta V Advantage isotope ratio mass spectrometer via a Thermo GC Combustion III (following Thornton et al. 2011). The ^{13}C-enrichment of the TOC and PLFAs were then determined from the $^{12}C/^{13}C$ ratios, following Hunter et al. (2013), correcting for background isotopic ratios of each material (*Supplementary tables 2 & 3*). All data were scaled to the areal cover (per mesocosm) by converting to values per cm^{-3} of wet sediment, pooling the data for from each sediment depth fraction (0-0.5; 0.5-2; 2-5; 5-10 cm) and normalised to total mesocosm area (0.24 m^2).

2.2 Data Analysis

We tested for significant interactions and independent effects of *Carcinus* presence and temperature by analysis of variance, treating *Carcinus* presence (2 levels: presence, absence) and temperature (2 levels: ambient, ambient + 2 °C) as fixed effects. We included water bath as a random error term within the ANOVA models to account for the potential effects of mesocosm location (Underwood, A. J. 1997; Crawley 2007). ANOVA model structure may, thus, be expressed as $y \sim C * T + \text{Error}(WB)$, where C = *Carcinus* presence, T = Temperature and WB is the Water Bath.

Data and residuals were visually explored to ensure they met assumptions of normality and homoscedacity (following Zuur, Ieno & Elphick 2010). We tested for the effects of *Carcinus* presence and temperature upon MPB biomass (sediment chlorophyll *a* concentrations) and the spatial variability of the MPB community independently during each four weeks of the experiment. The inclusion of *week* as a covariate would have required the extension of the ANOVA model to include a 3-way interaction term and a temporal autocorrelation structure. This was deemed inappropriate given the sample size within each our treatments (n=5).

We tested for treatment effects upon sediment TOC and TO^{13}C concentrations, total PLFA and ^{13}C-labelled PLFA concentrations as proxies for microbial biomass and microbial carbon retention at the end of the experimental incubations, and the relative abundance bacterial and microeukaryote fatty acid biomarkers (*Supplementary Table 4*) within both the total and ^{13}C-labelled PLFA pools. We tested for statistical significance at $p < 0.05$, as a reasonable compromise between the risk of false rejection of the null hypothesis (Type I error) and failure to detect a significant effect (Type II error), given the limited replication (n=5) within each treatment (Underwood, A. J. 1997). Where we detect a significant p-value, we calculated the effect size (η^2) as the sum of squares for any significant effect(s) / total sum of squares for the ANOVA model, following Cohen (1988). Effect sizes are reported alongside the p-values for each ANOVA in tables 1, 2 and 3. We consider a small effect size where $\eta^2 < 0.15$; with the associated p-values interpreted with caution (Cohen 1988). Data analysis were conducted in *R*, using the *base* and *Sciplot* packages (R Development Core Team 2009; Morales 2012). All data are publicly available through the Pangaea data repository, under a CC-BY creative commons license (https://doi.pangaea.de/10.1594/ PANGAEA.892199).

3. Results

Differences in the concentration and spatial variability of Chl_a within surficial sediments were tested independently during each of the four time points (weeks 0, 1, 2, 3 and 4), with *Carcinus* introduced to the mesocosms during week 1. *Carcinus* presence resulted in a significant decrease in MPB biomass during weeks 1 and 2 of the experiment, with no effects detected during weeks 0 and 3 (Fig 1a; Table 1a). Our data suggests a significant interaction between *Carcinus* presence and temperature affected MPB patchiness during week 1 (Fig 1b; Table 1b), albeit with a small effect

size (η^2 =0.112, Table 1b). No significant effects of *Carcinus* presence and temperature upon MPB patchiness were detected during weeks 2 or 3 (Fig 1b). We, thus, infer that *Carcinus* presence negatively affected the growth of the MPB during weeks 1 and 2 of the experiment. No significant effects of water bath, as a nesting factor, were detected upon either MPB biomass or patchiness.

We observed a significant effect of *Carcinus* presence upon sediment TOC concentrations (Fig. 2a; Table 2a), which decreased by ~ 15 % when crabs were absent. ^{13}C-label retention within the sediment (TO^{13}C) accounted for between 0.02 and 0.42 % of the ^{13}C-labelled bicarbonate dose added to each mesocosm (Fig. 2b; Table 2b). We identified a significant interaction between *Carcinus* presence and temperature on sediment TO^{13}C. *Carcinus* presence decreased sediment TO^{13}C concentrations but only under ambient conditions. Under the future warming conditions, by contrast, TO^{13}C concentration within the sediments was greater in the presence of *Carcinus*.

We used the concentrations of PLFAs in surficial sediments as a proxy for total microbial biomass. Total PLFA concentrations ranged between 11 and 26 mg C mesocosm^{-1}, equating to between 15 and 36 g microbial biomass within each mesocosm (following Middelburg et al., 2000; Evrard et al., 2010). Within this study, we detected no effects of either *Carcinus* presence or temperature upon microbial biomass (Fig. 2c; Table 2c). Microbial ^{13}C-retention was negatively affected by increased temperature (Fig. 2d; Table 2d).

In total, 31 individual PLFA were detected, of which five were identified as bacteria-specific fatty acids and three polyunsaturated fatty acids (PUFAs) as microeukaryote-specific biomarkers (*Supplementary Table 4*). Bacterial contributions to the PLFAs exhibited a significant increase in response to warming, with no effects of *Carcinus* presence (Fig 3a; Table 3a). Bacterial contributions to the ^{13}C-labelled PLFAs were affected by significant interactions between *Carcinus* presence and temperature (Fig. 3b; Table 3b). *Carcinus* presence reduced ^{13}C-labelling of the bacterial PLFAs under ambient conditions, and increased ^{13}C-labelling of the bacterial PLFAs under the warming treatment.

Significant interactions between *Carcinus* presence and warming affected the relative abundance of both the microeukaryote PUFAs (Fig. 3c; Table 3c) and their

contribution to the ^{13}C-labelled PLFA pool (Fig. 3d; Table 3d) in both cases *Carcinus* presence and warming resulted in significant decreases in PUFA concentrations and 13C-labelling relative to the ambient-no crab treatment. The most commonly detected polyunsaturated PLFA was 18:2ω6,9, which is widely recognised as a fungal biomarker (Stoeck et al. 2002; Kaiser et al. 2010; Frostegård, Tunlid & Bååth 2011), accounting for between 39.45 (± 1.81) % and 46.02 (± 2.69 %) of the total PUFAs.

4. Discussion

The study shows that the presence of the mobile epibenthic predator, *Carcinus maenas,* mediates the retention of newly fixed (^{13}C-labelled) carbon within intertidal sediments, with faunal impacts altered by changes to the ambient temperature. MPB biomass and patchiness, as (proxies for assessing the productive potential of coastal sediments, Hicks et al. 2009; Kahlert & McKie 2014), were both initially affected by the introduction of *Carcinus* to the mesocosms. Where *Carcinus* were present, the sediment surface was visibly disturbed with track marks, and appeared loss cohesive in texture. Alongside this disturbance the MPB biomass increased and MPB patchiness decreased over the subsequent two weeks, suggesting that faunal reworking of the sediment had little long term effects upon the MPB. This is surprising given that faunal disturbance tends to limit the accumulation of MPB biomass or fresh algal phytodetritus at the sediment surface (Canuel et al. 2007; Spivak et al. 2007; Hicks et al. 2009; Jeffreys et al. 2011; Fanjul et al. 2015). Benthic diatoms, however, can switch between autotrophy at the sediment surface and heterotrophic fermentation within oxygen and light limited sub-surface sediments (Bourke et al. 2016). We postulate that faunal reworking of the sediments provides a mechanism for the transfer of active MPB cells between the sediment surface and deeper sediment layers, which allows MPB biomass to recover from the initial disturbance event.

Coastal regions are important in the global carbon cycle, where organic matter burial exceeds 226 Tg C yr^{-1}, of which up to 50 % may be recycled back to the water column as carbon dioxide (Middelburg, Soetaert & Herman 1997; Bauer et al. 2013). Understanding how fauna influence these processes is critical if we are to predict the impacts of the current high rates of biodiversity loss. Within our study, the bulk of the sediment TOC pool consisted of non-labelled organic matter and can, thus, be considered analogous with ‘old’ carbon stored within the sediment. *Carcinus* absence

was associated with a small, but significant, decrease in the retention of this 'old' carbon within the sediment (Fig 2a). As an active bioturbator, *Carcinus* plays a key role reworking coastal sediments (Queiros et al. 2013). Given that sediment and crabs were collected from the same field site, it is reasonable to infer that sediment carbon stocks are in equilibrium with the presence of *Carcinus*. Consequently, the decrease in TOC associated with the absence of *Carcinus* suggests that faunal disturbance may limit microbial activity within the mesocosm sediments. This provides further evidence that benthic fauna, such as crabs, play an important role regulating microbial activity and the preservation of organic matter within coastal sediments (Mermillod-Blondin & Rosenberg 2006; Canuel et al. 2007; Spivak et al. 2007; Fanjul et al. 2015).

The ^{13}C-labelled organic matter represents a sedimentary carbon pool that has been "recently-fixed" by the MPB. This 'recently-fixed' carbon pool is likely to be composed of relatively labile short-chain organic molecules (e.g. lipids, amino acids), which may be preferentially utilised as a carbon source by the sediment microbial community (e.g. van Nugteren et al. 2009; Miyatake et al. 2014). Our study identified contrasting effects of *Carcinus* presence upon the concentrations of this 'recently-fixed' carbon under both the present and future warming conditions (Fig. 2b). No significant differences in surficial MPB biomass were observed during the isotope-labelling phase of the experiment (Fig. 1a) and, under ambient conditions, *Carcinus* presence had no effect upon ^{13}C-fixation into the organic matter pool (Fig. 2B) Consequently, crab activity had no effect upon C-fixation by the MPB contrasting with previous studies that have identified faunal disturbance of the sediment surface as an important control upon microbial carbon fixation (Mermillod-Blondin & Rosenberg 2006). Under the predicted future-warming treatment, however, the absence of *Carcinus* is associated with reduced retention of ^{13}C-labelled carbon in the sediment. This indicates that under the predicted warming conditions tested, increased metabolic activity by the sediment microbial community, leading to more rapid fixation of the ^{13}C-label by the MPB and more rapid mineralisation of 'recently-fixed' carbon by heterotrophic microorganisms (Gillooly et al. 2001; Weston & Joye 2005; Daufresne et al. 2009; Morán et al. 2015).

The microbial response to *Carcinus* presence and warming were tested in the surficial sediment layer (0-0.5 cm), using PLFAs as biomarkers. PLFAs provide a powerful

tool in this context, allowing both microbial biomass and incorporation of the ^{13}C-label to be quantified (Middelburg et al. 2000; Mayor et al. 2012; Hunter et al. 2013). Microbial biomass (estimated from PLFA concentrations) was unaffected by either *Carcinus* presence or temperature (Fig. 2c), however, we observed a significant decrease in ^{13}C-label retention within the PLFAs (Fig 2d). This is likely to reflect increased metabolic activity within the microbial community as a response to ocean warming (Weston & Joye 2005). *Carcinus* presence played no significant role in determining ^{13}C-label retention within the PLFAs. We suggest that turnover of microbial biomass at the sediment surface (reviewed in Mermillod-Blondin & Rosenberg 2006), combined with temperature-dependent increases in microbial metabolism (Weston & Joye 2005; Arndt et al. 2013) may explain this observation.

Our results show that faunal species loss has pronounced effects upon microbial community responses to ocean warming. In this context, *Carcinus* presence had a significant influence upon both the structure of the microbial community and its active component, as revealed from the relative abundance of bacteria-specific FAs and PUFAs within the total PLFA and ^{13}C-labelled PLFA profiles. PLFAs can provide useful information on microbial community structure in aquatic sediments (e.g. Stoeck et al. 2002; Boschker et al. 2005; Mayor et al. 2012), albeit with caveats regarding their limited taxonomic resolution (Frostegård et al. 2011). We observed broad trends in the response of the microbial community within our mesocosms. This is characterised by temperature-driven decreases in the bacterial FAs, which reflect the impacts of ocean warming on microbial cell-size (Morán et al. 2015). There was a strong negative effect of crab presence upon the PUFAs under ambient conditions. PUFAs can be considered both as a biomarker of microeukaryote biomass (Stoeck et al. 2002; Kaiser et al. 2010; Frostegård et al. 2011) and an indicator of the relative lability of the sediment organic matter pool (Canuel et al. 2007; Spivak et al. 2007). Within our study, PUFAs were dominated by a single fungal biomarker (18:2ω7) suggesting that, under ambient conditions *Carcinus*, may suppress the accumulation of fungal biomass within surficial sediments. By contrast, PUFA concentrations were lower under future warming conditions, perhaps as a consequence of increases in resource competition and biomass turnover within the microbial community (Daufresne et al. 2009; Sarmento et al. 2010).

The active microbial community, as revealed by ^{13}C-labelling of the PLFAs, were sensitive to changes in *Carcinus* presence under both ambient and future-warming conditions. Both bacterial and microeukaryote biomarkers exhibited similar responses, with *Carcinus* presence associated with decreased contribution to the active microbial community under ambient conditions, but increasing their contributions under warming conditions. Our study builds upon previous studies which demonstrate that crabs play a key role in coastal sediments, controlling accumulation of microbial biomass and the preservation of organic matter (Canuel et al. 2007; Spivak et al. 2007; Fanjul et al. 2015). This reinforces the concept of the benthic fauna as a 'gearbox' that regulates microbial activity in marine sediments (van Nugteren et al. 2009; Hunter et al. 2012) and support the observation that the responses of faunal communities to rising ocean temperature will have cascading effects upon benthic primary producers (O'Connor et al. 2009; Mrowicki & O'Connor 2015).

Retention of the ^{13}C-label within the mesocosm sediment was low compared with other studies that investigated label uptake by the MPB (e.g. Middelburg et al. 2000; Boschker et al. 2005; van Oevelen et al. 2006). This reflects the relatively low dose of ^{13}C-labelled sodium bicarbonate introduced into each mesocosm, and potential losses associated with washout of unused ^{13}C-labelled sodium bicarbonate; mineralisation of ^{13}C-labelled organic matter and flux of dissolved organic carbon between the sediment and overlying water (e.g. Middelburg et al. 2000; Evrard et al. 2010). Given that coastal sediments are typically respiration-dominated systems (Woulds et al. 2009; Woulds et al. 2016), mineralisation of ^{13}C-labelled organic matter is likely to represent a major un-quantified process within our study. Caution is therefore advised in comparing these data with other sediment carbon budgets, derived using similar methodologies (Middelburg et al. 2000; Evrard et al. 2010; Woulds et al. 2016). Our study builds upon observations of cascading effects of crab predation upon sediment organic matter composition in coastal sediments (Canuel et al. 2007; Spivak et al. 2007; Fanjul et al. 2015), and provides a direct test of how faunal species loss affects sediment carbon sequestration under both present climatic conditions and predicted future-warming conditions.

Our study demonstrates that both faunal presence and temperature are important regulators organic matter retention within coastal sediments. Faunal presence was the

primary driver of changes in 'old' organic carbon within the mesocosm, whilst strong interactions between faunal presence and temperature determine the fate of 'recently-fixed' organic matter. Bulk analysis of the TOC and total PLFAs was, however, limited in its scope to identify the interacting effects of *Carcinus* presence and temperature. Compound-specific analysis of the PLFA profiles, allowed us, to discern how faunal presence stimulated ^{13}C-incorporation by heterotrophic microbes under the predicted-future warming treatment, and suppressed the accumulation of microeukaryote (fungal) biomass under ambient conditions. These effects are clearly driven by changes in faunal bioturbation and the rate-limiting effects of temperature upon microbial activity.

We acknowledge that the results of mesocosm experiments are largely illustrative, and cannot easily be scaled to natural systems (Oviatt 1994; Carpenter 1996; Queiros et al. 2015). If we are to mitigate the impacts of global biodiversity loss, however, manipulative experiments are an important tool to test potential impacts on ecosystem processes (Duffy 2009; Cardinale et al. 2012; Stewart et al. 2013; Donohue et al. 2017). Previous mesocosm studies highlight the importance of epibenthic predators, such as crabs, as mediators of organic matter preservation in coastal sediments (Canuel et al. 2007; Spivak et al. 2007; Atwood et al. 2015; Donohue et al. 2017; Fanjul et al. 2015). Here we demonstrate that under future warming conditions, epibenthic predator loss leads to an increase in the relative importance of microbial C cycling pathways in coastal sediments. Whilst we cannot easily predict ecosystem-scale responses, this may result in decreasing carbon storage within coastal sediments. Consequently the combined effects of predator loss and ocean warming are likely to have adverse effects upon a range of coastal ecosystem services that are relevant for climate regulation, waste processing, flood protection and support of fisheries (reviewed in Lopes & Vidiera 2013; Isbell et al. 2017).

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Author Contributions

Experiments were designed by WRH and NO'C, and conducted by WRH. Analytical work was carried out by WRH and NO. WRH, NO and NO'C all contributed to the writing of the manuscript.

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Tables Legends

Table 1. ANOVA summary tables of the effects of *Carcinus* presence and temperature upon a) MPB Biomass (Chlorophyll a) and b) the spatial variability of the MPB during each of the four weeks of the experiment. *Abbreviations: T = Temperature; C = Carcinus; WB = Water Bath.*

a) MPB Biomass (Chlorophyll *a*)

Week 0

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.199	0.199	4.526	0.050	-
C	1	0.002	0.002	0.045	0.835	-
T x C	1	0.014	0.014	0.323	0.578	-
Resid.	15	0.661	0.044			

Error

	df	**SS**	**MS**
WB	1	0.271	0.271

Week 1

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.028	0.028	0.787	0.389	-
C	1	0.353	0.353	9.951	**0.007**	**0.298**
T x C	1	0.001	0.001	0.004	0.948	-
Resid.	15	0.532	0.036			

Error

	df	**SS**	**MS**
WB	1	0.271	0.271

Week 2

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.019	0.019	0.268	0.612	-
C	1	0.953	0.953	13.439	**0.002**	**0.359**
T x C	1	0.124	0.124	1.741	0.207	-
Resid.	15	1.064	0.071			

Error

	df	**SS**	**MS**
WB	1	0.496	0.496

Week 3

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.129	0.129	1.165	0.298	-
C	1	0.299	0.299	2.711	0.120	-
T x C	1	0.011	0.011	0.104	0.752	-
Resid.	15	1.656	0.110			

Error

	df	**SS**	**MS**
WB	1	0.016	0.0163

b) MPB Patchiness

Week 0

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.001	0.001	0.127	0.726	-
C	1	0.003	0.003	0.347	0.565	-
T x C	1	0.005	0.005	0.521	0.482	-
Resid.	15	0.149	0.009			

Error

	df	**SS**	**MS**
WB	1	0.003	0.003

Week 1

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.019	0.019	1.353	0.263	-
C	1	0.255	0.255	17.997	**<0.001**	**0.447**
T x C	1	0.064	0.064	4.524	**0.049**	**0.112**
Resid.	15	0.212	0.014			

Error

	df	**SS**	**MS**
WB	1	0.021	0.021

Week 2

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.043	0.043	1.337	0.266	-
C	1	0.015	0.015	0.471	0.503	-
T x C	1	0.001	0.001	0.037	0.851	-
Resid.	15	0.485	0.032			

Error

	df	**SS**	**MS**
WB	1	0.024	0.024

Week 3

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.003	0.003	0.090	0.768	-
C	1	0.008	0.008	0.273	0.609	-
T x C	1	0.104	0.104	3.728	0.073	-
Resid.	15	0.420	0.028			

Error

	df	**SS**	**MS**
WB	1	0.001	0.001

Table 2. ANOVA summary tables of the effects of *Carcinus* presence and temperature upon the sediment a) TOC, b) TO^{13}C, c) PLFA, d) ^{13}C-labelled PLFA concentration. *Abbreviations: T = Temperature; C = Carcinus; WB = Water Bath.*

a) Sediment TOC concentrations

	df	SS	MS	F	*p*	η^2
T	1	11.140	11.145	3.585	0.078	-
C	1	15.330	15.328	4.930	**0.042**	**0.198**
T x C	1	0.020	0.021	0.007	0.935	-
Resid.	15	46.630	3.109			

Error

	df	SS	MS
WB	1	4.144	4.144

b) Sediment TO^{13}C concentrations

	df	SS	MS	F	*p*	η^2
T	1	2105	2105	5.107	**0.039**	**0.149**
C	1	103	103	0.249	0.625	-
T x C	1	4939	4939	11.984	**0.003**	**0.352**
Resid.	15	6183	412			

Error

	df	SS	MS
WB	1	706	706

c) Total PLFAs

	df	SS	MS	F	*p*	η^2
T	1	0.296	0.297	2.806	0.115	-
C	1	0.006	0.006	0.053	0.822	-
T x C	1	0.166	0.166	1.573	0.229	-
Resid.	15	1.581	0.105			

Error

	df	SS	MS
WB	1	0.362	0.362

d) ^{13}C-labelled PLFAs

	df	SS	MS	F	*p*	η^2
T	1	45950	45950	20.887	**<0.001**	**0.472**
C	1	1776	1776	0.807	0.383	-
T x C	1	6187	6187	2.812	0.114	-
Resid.	15	33000	2200			

Error

	df	SS	MS
WB	1	10530	10530

Table 3. ANOVA summary tables of the effects of *Carcinus* presence and temperature upon the relative contribution of a) bacterial fatty acids, b) ^{13}C-labelled bacterial fatty acids, c) microeukaryote fatty acids PUFAs and d) ^{13}C-labelled PUFAs, to the total PLFA and ^{13}C-labelled PLFA pools. *Abbreviations: T = Temperature; C = Carcinus; WB = Water Bath.*

a) Bacterial FAs

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	25.96	25.956	10.942	**0.005**	**0.301**
C	1	1.56	1.565	0.660	0.429	-
T x C	1	6.61	6.609	2.786	0.115	-
Resid.	15	35.58	2.372			

Error

	df	**SS**	**MS**
T	1	16.46	16.46

b) ^{13}C-labelled Bacterial FAs

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	315.5	315.52	7.888	**0.013**	**0.249**
C	1	17.5	17.49	0.437	0.519	-
T x C	1	244.8	244.77	6.120	**0.026**	**0.194**
Resid.	15	600.0	40.00			

Error

	df	**SS**	**MS**
T	1	86.2	86.2

c) PUFAs

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.609	0.609	40.77	**<0.001**	**0.117**
C	1	2.421	2.421	162.02	**<0.001**	**0.463**
T x C	1	1.644	1.644	110.02	**<0.001**	**0.315**
Resid.	15	0.224	0.015			

Error

	df	**SS**	**MS**
Temp	1	0.329	0.329

d) ^{13}C-labelled PUFAs

	df	**SS**	**MS**	**F**	***p***	η^2
T	1	0.539	0.539	1.559	0.231	-
C	1	2.806	2.806	8.120	**0.012**	**0.167**
T x C	1	7.533	7.533	21.796	**<0.001**	**0.447**
Resid.	15	5.184	0.346			

Error

	Df	**SS**	**MS**
T	1	0.779	0.779

Figures

Figure 1. Effects of *Carcinus* presence and temperature upon the microphytobenthos. Temporal changes in mean (± standard error) a) MPB Biomass (surficial chlorophyll a concentrations) and b) spatial variation in the MPB assemblage between *Carcinus* present (white + 🦀) and absent (grey) treatments, under both ambient (🌡) and warming (🌡) temperature treatments. Inserts show pooled means (± standard error) where significant independent effects of either *Carcinus* presence (white) / absence (grey) or temperature (labelled) where detected. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Where significant interactions were identified, groups labeled with the same lowercase letter are not significantly different ($p > 0.05$; Tukey's tests).

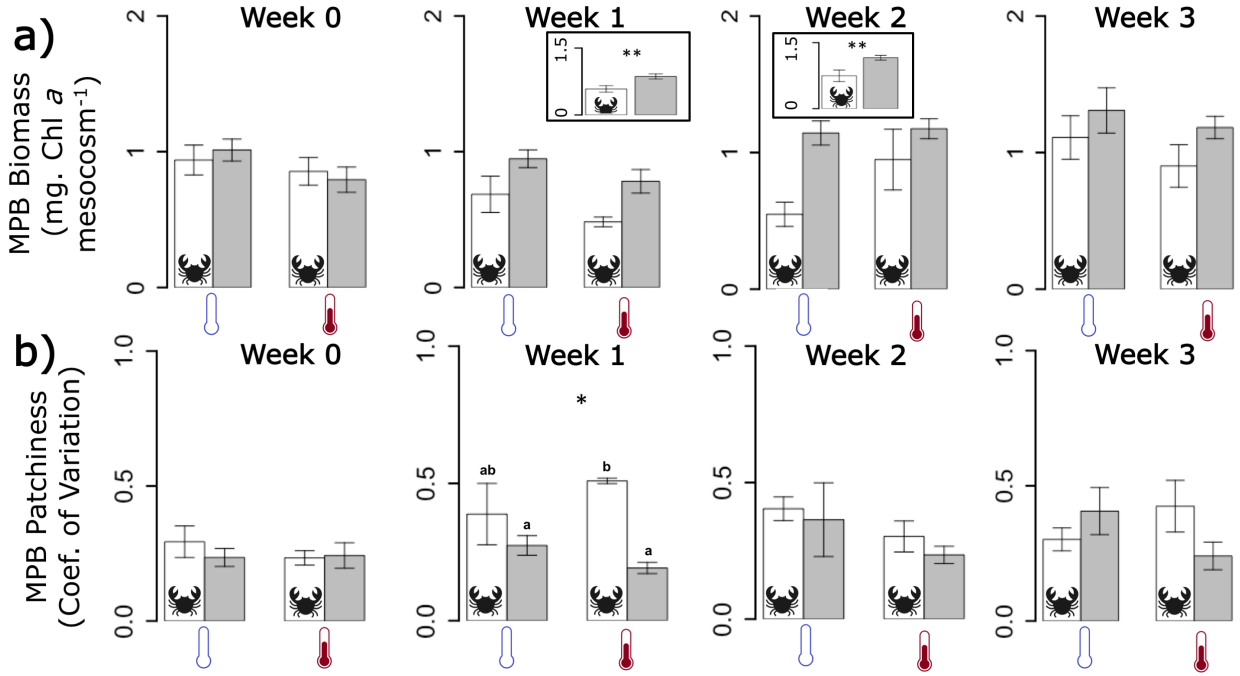

Figure 2. Effects of Carcinus presence and temperature upon the sediment geochemistry. Mean (± standard error) concentrations of a) Total Organic Carbon b) ^{13}C-labelled Organic Carbon (TO^{13}C) c) Total PLFAs and d) ^{13}C-labelled PLFAs between *Carcinus* present (white + 🦀) and absent (grey) treatments, under both ambient (🌡) and warming (🌡) temperature treatments. Inserts show pooled means (± standard error) where significant independent effects of either *Carcinus* presence (white) / absence (grey) or temperature (labelled) where detected. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Where significant interactions were identified, groups labeled with the same lowercase letter are not significantly different ($p > 0.05$; Tukey's tests).

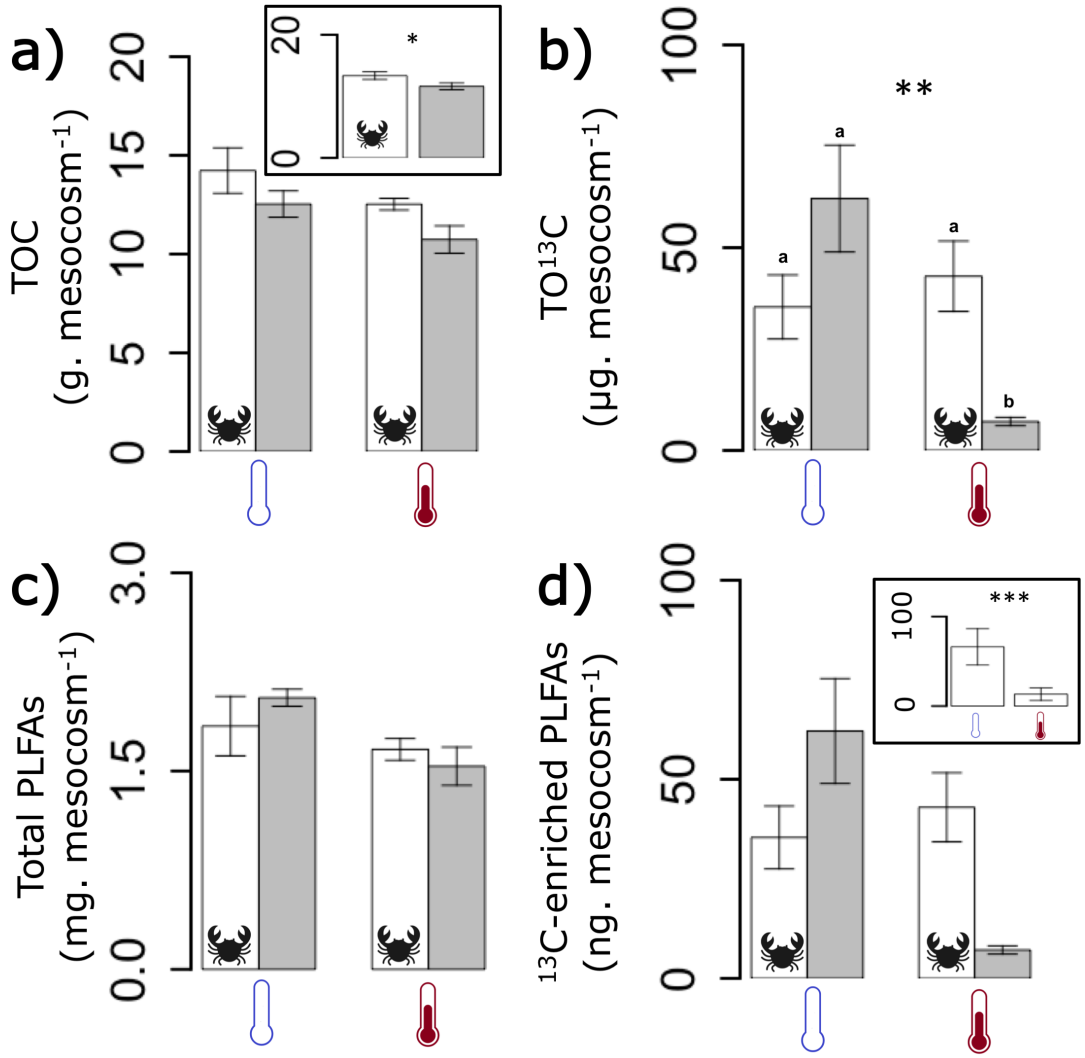

Figure 3. Effects of Carcinus presence and temperature upon the sediment microbial community. Mean (± standard error) contributions of a) bacterial fatty

acids b) ^{13}C-labelled bacterial Fatty Acids, c) microeukaryote PUFAs and d) ^{13}C-labelled microeukaryote PUFAs [relative to the total PLFA or ^{13}C-labelled PLFA pools] between *Carcinus* present (white + 🦀) and absent (grey) treatments, under both ambient (🌡) and warming (🌡) temperature treatments. Inserts show pooled means (± standard error) where significant independent effects of either *Carcinus* presence (white) / absence (grey) or temperature (labelled) where detected. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Where significant interactions were identified, groups labeled with the same lowercase letter are not significantly different ($p > 0.05$; Tukey's tests).